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Studies on populations of *Folsomia candida* (Insecta: Collembola): causes of aggregations

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With one figure

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1. Introduction

In laboratory studies on populations of insects, the population density is calculated as the number of insects per culture or per unit of area or volume of the whole culture vessel. Using *Folsomia candida* (Willem), Usher et al. (1971) recorded population densities as the number of insects per unit of area, but they frequently observed that individuals of *F. candida* aggregated. Thus, within the culture some areas had a high population density, whilst other areas were more or less without insects. This poses the question of whether some regulatory mechanism is maintaining the population of Collembola at some more or less fixed population density locally within the area occupied by the insects irrespective of the average population density for the whole culture.

The habit of *F. candida* in forming aggregations in cultures has also been observed by Green (1964), and many field studies (for example those of Glasgow1939, Poole 1961, 1962, 1964, and Usher 1969) have shown that Collembola are normally aggregated in the soil. However, although the detection of aggregation has been the aim of many studies, the factors causing these aggregations have less frequently been studied. The studies where the causes have been investigated (for example Poole 1962 and Usher 1967) have concentrated upon estimating correlation coefficients between numbers of Collembola in a sample and various facets of the micro-environment, and Usher (1969) has attempted to relate the size and number of aggregations in field populations to the average population density. A review of the factors causing aggregations of soil arthropods in the field is given by Butler et al. (1971).

Although there is a body of field evidence, controlled laboratory investigation of aggregation responses have not in general been performed. One exception is that of Christiansen (1970) who observed 15 species in the laboratory and measured their aggregation on a clay substrate. He further subjected 13 species, including *F. candida*, to a multiple choice experiment whereby the Collembola could choose sand, clay or earth substrates, each under wet and dry conditions. There is considerable evidence (Snider 1972) to suggest that tempera-

ture has an effect upon *F. candida*, particularly in relation to the speed of growth, maturation and egg production.

2. Methods

All stock cultures of *F. candida* were maintained in cylindrical plastic chambers, as described by USHER et al. (1971). Individuals of *F. candida* were shaken from the stock cultures onto a water surface, and were transferred from this to the experimental boxes by a soft brush.

For all of the experiments perspex boxes, 23 cm square and 7 cm deep, were used. A floor of Plaster of Paris was cast in these boxes to a depth of approximately 1 cm, and this was covered with approximately 2 cm of gravel. The whole was kept damp with distilled water, and the boxes were kept in the dark at a temperature of approximately 20 °C. A sampling tool, consisting of a square grid of stainless steel blades, was used to cut a block of sixteen square cores each of side 4 cm. The insects were extracted from the cores by flotation in a magnesium sulphate solution of density 1.185, during which the mixture was stirred to release Collembola from air pockets.

In the multifactor experiment four sets of factors were investigated, namely (i) subtrate, (ii) population density, (iii) time and (iv) initial pattern:

(i). As a substrate, two gravel grades were used, one of large particles in the size range 2.0 to 5.0 mm (giving a pore space of approximately 44 per cent) and one of small particles in the size range 0.5 to 2.0 mm (pore space approximately 38 per cent). In both substrates *F. candida* was able to live and move, and individuals placed on the surface quickly burrowed away from the light.

(ii). Three population densities were used, corresponding to average population densities of 1, 4 and 16 *F. candida* per 16 cm² of surface area (these corresponded to 33, 132 and 528 insects per box respectively). These population densities were chosen since they related to a range of intense aggregation noticed in the cultures being used by USHER et al. (1971).

(iii). The experiments were performed over three periods of time, this being defined as the time elapsing between the introduction of *F. candida* to the box and the cutting of the sixteen samples. The times used were 1 hour, 1 day and 1 week. The shorter time was included so as to investigate the reactions of insects that had recently been disturbed, whilst the longer time would be likely to indicate conditions in a more stable environment.

(iv). In order to investigate the effect of the initial pattern of distribution of *F. candida*, the gravel surface was divided into a grid of 30 equal compartments, and numbers of *F. candida* were added to each grid compartment using a known distribution. Thus, for an uniform distribution, 27 of the compartments received 1 insect whilst three, at random locations, each had two insects giving a total of 33 insects or a population density of 1 per 16 cm². Besides using an uniform distribution the initial patterns employed were also random, weakly aggregated and strongly aggregated distributions, as indicated in Table 1.

Table 1. Data on the initial patterns for *Folsomia candida* in the multifactor experiment

Distribution	Density per 16 cm²	Indices χ^2	$\overset{*}{m}$	$\overset{*}{p}$
Uniform	1	2.45	0.18	0.165
	4	1.63	3.46	0.785
	16	0.41	16.61	0.944
Random	1	29.73	1.09	0.990
	4	23.90	4.20	0.954
	16	18.31	17.21	0.977
Weakly aggregated	1	44.27	1.58	1.433
	4	46.63	4.95	1.124
	16	44.27	18.08	1.027
Strongly aggregated	1	60.64	2.12	1.938
	4	68.45	5.68	1.290
	16	61.65	18.66	1.059

Three indices of aggregation were employed. The χ^2 criterion, described by BLISS and FISHER (1953), is

$$\chi^2 = \frac{s^2}{m}(n-1)$$

where m is the mean, s^2 the variance and n the sample size. This index has been used to investigate aggregations of Collembola in the field by USHER (1969). The index of mean crowding (LLOYD, 1967), is

$$\overset{*}{m} = \frac{\sum x_i^2}{\sum x_i} - 1$$

where x_i is the number of *F. candida* in the ith sample ($i = 1, 2, \ldots, n$). $\overset{*}{m}$ is defined as the mean number of other individuals per individual in a sample, and the size of $\overset{*}{m}$ is therefore affected by the size of m, the mean number of insects per sample. The third index used, the index of mean patchiness (Lloyd 1967), is

$$\overset{*}{p} = \overset{*}{m}/m$$

where m and $\overset{*}{m}$ are defined above is an attempt to eliminate the effect of the mean from the index of aggregation. The values of these three indices of aggregation calculated for the initial distributions are shown in Table 1. It will be seen that these particular initial distributions were selected such that the values of the χ^2 criterion (with 29 degrees of freedom) for the uniform, random, weakly aggregated and strongly aggregated distributions were near to the tabulated values for $p = 0.05$, $p = 0.50$, $p = 0.95$ and $p = 0.99$ respectively.

Three sets of subsidiary experiments were also carried out. These experiments, designed to investigate the effect of single factors on the pattern of aggregation, concerned the reaction of the Collembola to water, food and long straight edges. Details of the design of each of these experiments are given with the results.

3. Results

3.1. The multifactor experiment

It was apparent that fewer Collembola were extracted from the samples than had been originally placed on the gravel. Thus, with the introduction of 1 insect per 16 cm², a mean in the samples of 0.84 per 16 cm² was observed after 1 hour, 0.93 after 1 day and 0,83 after 1 week. These means are not significantly different from each other, but their average of 0.87 per 16 cm² is significantly less than the original mean of 1 per 16 cm² ($t = 2.460$, $0.01 < p < 0.05$). Similarly with initial densities of 4 and 16 per 16 cm² the means after 1 hour, 1 day and 1 week are not significantly different, but their averages of 2.84 and 8.87 per 16 cm² are significantly less than the initial densities ($t = 5.290$, $p < 0.001$ and $t = 12.770$, $p < 0.001$ respectively).

The analysis of variance of the χ^2 criterion for estimating the degree of aggregation (Table 2) shows that there were no significant differences between the two gravel types or between the four starting distributions. The mean number of aggregations per grid of samples were 0.42, 0.62, and 1.38 after 1 hour, 1 day and 1 week respectively. At starting densities of 1, 4 and 16 Collembola per 16 cm² there were 0.21, 0.79 and 1.42 aggregations per grid of samples respectively.

Table 2. The analysis of variance of the χ^2 criterion for assessing the degree of aggregation in the multifactor experiment

Source	d.f.	S.S.	M.S.	F and Significance	
Gravel types	1	96.30	96.30	<1	n.s.
Time period before sampling	2	275.36	1375.68	7.69	$p \doteq 0.001$
Initial density	2	4634.16	2317.08	12.95	$p < 0.001$
Initial distribution	3	112.33	37.44	<1	n.s.
Error	63	11276.54	178.99		
Total	71	18870.68			

A linear relation between mean crowding and mean density has been postulated by Iwao (1968), and such a relation has been shown in field populations of Collembola by Takeda (1973). In these three analyses of variance there is a significant difference between the three time periods. It was therefore decided to apply Iwao's regression analysis to the data for 1 hour, 1 day and 1 week, grouping data irrespective of gravel type or initial distribution (neither of which had shown significant differences with any of the indices of aggregation) or of the initial population density (since a range of densities was required for the regression).

The regression equation for data collected after 1 hour is

$$\overset{*}{m} = -0.016 + 1.091\ m$$

after 1 day is

$$\overset{*}{m} = 0.048 + 1.154\ m$$

and after 1 week is

$$\overset{*}{m} = 0.767 + 1.104\ m$$

In all three of these equations the regression coefficient is significantly different from zero ($p < 0.001$ in all cases), but in no case is the regression coefficient significantly greater than 1, the regression coefficient that IWAO (1968) showed would be expected with a Poisson (random) distribution. Thus, although aggregations can be demonstrated in the laboratory cultures, the aggregations are not as strong as those shown to occur in field populations of Collembola.

3.2. Water experiment

Distilled water was allowed to drop from a burette at a rate of 1 drop every 30 seconds into the centre of a box containing the large gravel grade. The density of Collembola was 16 per 16 cm^2, and they were introduced in an uniform pattern. After 1 hour, before the culture medium had become saturated with water, the sampling tool was pushed in, the centre of the tool corresponding in position to the location of the water drop.

Six such experiments were carried out, two with Collembola that had been fed and watered regularly, two with Collembola that had had no food for four weeks but had had regular fresh water, and two with Collembola that had been given no fresh food or water for four weeks. For the purposes of analysis, all the Collembola were assumed to be at the centre of the sample in which they were found, and thus there are three horizontal distances between the sample centre and the drop's position, as shown in Fig. 1.

A regression was carried out on the number of Collembola in a sample as a function of the sample's distance from the water drop.

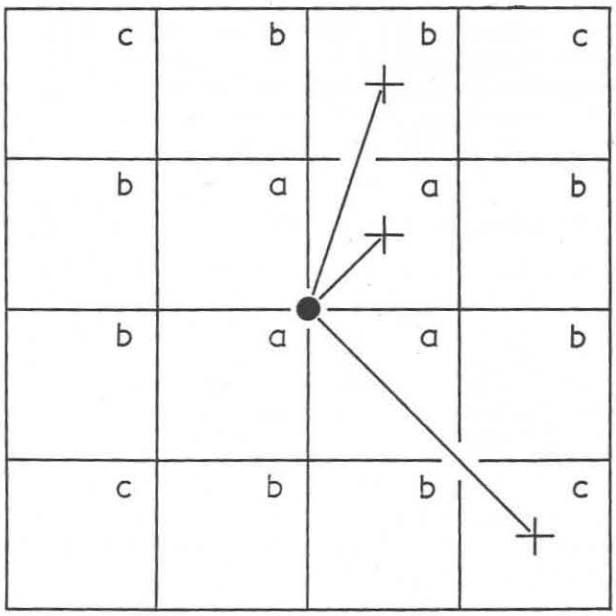

Fig. 1. A representation of a grid of samples. The squares labelled "a" are closest to water or food location (horizontal distance 2.828 cm), the "b" squares are intermediates (distance 6.325 cm) and the "c" squares are furthest (distance 8.485 cm).

The equation for the Collembola that had had regular food and water was

$$N = 27.106 - 3.351\ D$$

where N is the number of Collembola and D is the horizontal distance of the sample's centre from the water drop. The other equations were

$$N = 26.268 - 3.112\ D$$

and

$$N = 27.469 - 3.282\ D$$

for Collembola with water and no food and for Collembola with neither water nor food respectively.

In all cases the regression coefficient was significantly different from zero ($p < 0.001$), and an analysis of covariance (FREESE 1964) shows there to be no significant differences between the slopes or the levels of these equations. The negative sign to the regression coefficient indicates that the Collembola are aggregating in the vicinity of the water drop.

3.3. Food experiment

The Collembola used in these experiments had been given no fresh food for a period of four weeks prior to the experiment taking place, though they had received fresh water during this period. Twelve experiments were performed, three at a density of 1 insect per 16 cm² with 0.2 g of yeast, three at a density of 4 per 16 cm² with 0.2 g of yeast, and six at a density of 16 per 16 cm² (three of these with 0.1 g and three with 0.2 g of yeast). In each group of three, one experiment was terminated after 1 hour, one after 1 day and one after 1 week. The yeast was placed in the centre of the culture box, the large gravel grade was used, and the Collembola were introduced in an uniform pattern. The location of the yeast was used to position the centre of the sampling tool when the experiment was concluded.

The results of fitting regression equations to relate the number of Collembola in a sample to the distance of the sample's centre from the yeast are shown in Table 3. In general it will be seen that the Collembola quickly aggregated around the food source, and that the aggregations were maintained probably until after the food source had been eaten out. Covariance analysis indicated that the three regression coefficients for each density and food level were significantly different.

Table 3. The values of the regression coefficients, b, in the equation $N = a + bD$ where N is the number of Collembola and D is the horizontal distance from the food source

Density of Collembola (No. per 16 cm²)	Weight of yeast (g)	Period of time		
		1 hour	1 day	1 week
1	0.2	— 0.418**	— 0.478**	— 0.018 n.s.
4	0.2	— 0.693**	— 0.705**	— 0.562*
16	0.2	— 12.938***	— 9.965**	— 1.940*
16	0.1	— 0.284 n.s.	— 4.943***	— 0.533 n.s.

The significance is indicated by n.s. — not significant, * — $0.05 > p > 0.01$, ** — $0.01 > p > 0.001$, *** — $P < 0.001$.

3.4. Straight edge experiments

Since Collembola in culture vessels have a tendency to collect around the edge, four experiments were performed to assess the effect of short straight edges within the culture medium. A piece of perspex 8 cm long and about 2 cm deep was placed with its centre in the centre of the culture box, which contained coarse gravel and Collembola at a density of 16 per 16 cm² introduced in an uniform pattern. After 1 day the perspex edge was pulled out and the sampling tool quickly pushed in. In only one of the four experiments were there significantly more Collembola in the samples beside the perspex edge (mean for edge samples

= 7.00, mean for other samples = 3.71, $F = 10.64$, $0.01 < p < 0.05$) than in the sample away from the edge. However, in the other three experiments the mean number of Collembola in samples by the edges were greater, but not significantly so, than the mean number in samples away from the edge (3.00 and 1.92, 3.75 and 2.83, 7.50 and 5.50 respectively).

4. Discussion

In the laboratory, a criterion of culturing conditions has often been that the Collembola must be visible, and thus techniques have been developed for culturing Collembola on a floor of a mixture of charcoal and Plaster of Paris. These methods obviously influence the behaviour of the Collembola since they force the insect the live in a two rather than a three-dimensional environment. The present experiments were undertaken in an attempt to investigate the three-dimensional behaviour of Collembola in the laboratory. However, there is still one important difference between the field situation and these laboratory experiments. All of the experiments were carried out in square boxes of area 529 cm^2, so that the field for movement was bounded in all directions. An allowance was made for this by using a square sampling tool of area 256 cm^2 so that only 48 per cent of the experimental field, away from the boundaries, was sampled.

In the multifactor experiment, Collembola were distributed in an uniform, random and in two aggregated types of pattern over the entire surface of the experimental field. The results of analyses of indices of aggregation showed that this initial pattern of distribution did not affect the resultant pattern of aggregations. It is therefore clear that the Collembola did not remain in the place where they were introduced, but that they distributed themselves in some other way in the culture medium. The fact that there was no significant difference between the two gravel types indicates that the structure of the environment was not important, though the two gravel types were sufficiently large for there to be no physical barriers to the penetration of the Collembola.

Three results need discussion. First, although the Collembola were distributed over the entire surface of the experimental field, sampling only gave an average recapture of 59.9 per cent. Other observations indicated that the 40.1 per cent not recaptured would mostly have migrated to the edges of the culture boxes and would not represent the mortality of the population (which would probably be less than 2 per cent over a period of 1 week). The reasons why the edges of the culture box might prove attractive are discussed below. Secondly, the strength of the aggregations depends on the population density, since the indices calculated for initial densities of 16 per 16 cm^2 are the largest. It would therefore appear that some kind of mutual attraction between insects can give rise to aggregation, and this is supported by observational data that there might be an olfactory stimulus. Thirdly, time was an important factor. After 1 hour the mean number of aggrations detected in a grid of samples was 0.125 at initial density of 1 per 16 cm^2 and 0.875 at an initial density of 16 per 16 cm^2, whereas after 1 week these mean numbers of aggregations had risen to 0.375 and 2.125 respectively. After the disturbance of transferring *F. candida* to this new culture medium, time and a sufficient number of Collembola were needed before aggregations became established.

In this experiment the culture medium was as homogeneous as possible, no food and no water being added after the Collembola had been introduced. However, the Collembola often aggregated along the edges, and so possible factors which determine the location of aggregations were investigated. It was shown that aggregations formed around sources of both food and water, irrespective of whether or not the Collembola had been in contact with a plentiful supply of either or both immediately prior to being transferred to an experimental situation. The location of food and fresh water, probably in the form of droplets of condensation, therefore appear to be important in determining the location of aggregations. The effect of edges is less clear. These are unlikely to induce a thigmotactic reaction in the Collembola since the gravel particles would themselves be sufficiently large. However,

they do present a barrier to an individual that is moving. It is a relatively common feature of laboratory insects that they stop and wait for a while after they meet a barrier (the effect of barriers on the distribution of organisms in cultures has been discussed and modelled by USHER and WILLIAMSON 1970). Barriers also provide an area where, due to temperature differences across the barrier, water tends to condense, and hence it might be the water that results in the Collembola being more abundant in the vicinity of edges.

The dynamics of aggregations of soil arthropods have been discussed by USHER (1969) who distinguished three types which can be defined thus:

Type I, as the population density increases so the number of aggregations increases proportionally.

Type II, as the population density increases so the number of insects within each aggregation increases proportionally.

Type III, is the compound of the other two types in that as the population density increases so both the number of aggregations and the number of insects within an aggregation increase.

These can be detected by the correlations between the population density and the number of aggregations (r_n) and the mean number of insects per aggregation (r_s). In studying field populations of Collembola the data were so variable that the two correlation coefficients were often not significant. However, to determine the aggregation type of *F. candida* a series of ten experiments were set up in an experimental field 16 cm by 16 cm, of coarse gravel, the whole of which was sampled. Experiments were initiated with densities of 1, 2, 4, 8 and 16 indiciduals per 16 cm^2, and were left for 1 week before sampling.

Table 4. Data derived from 10 experiments to determine the relation between population density (D) and the number (N) and size (S) of aggregations

Population density (No. per 16 cm^2)	Number of aggregations	Mean number of Collembola per aggregation
0.8125	0	0
1.0	1	4.0
2.0	1	13.0
2.0	1	9.0
4.0	2	11.5
4.0	3	16.0
8.0	4	23.0
8.0	2	20.5
15.3125	3	42.3
16.0	5	39.4

The data are shown in Table 4 where it will be seen that mortality and sampling inefficiency was only 1.39 per cent. From this data it was determined that

$$N = 0.825 + 0.225\ D\ (r_n = 0.819)$$

and

$$S = 3.107 + 2.416\ D\ (r_s = 0.976)$$

where D is the density (Collembola per 16 cm^2), N is the number of aggregations formed and S is the number of Collembola in an aggregation. Since both r_n and r_s are positive and significant, it can be concluded that in the laboratory *F. candida* forms a type III aggregation.

It would appear that *F. candida* forms its aggregations at sites which are particularly favourable for food or water. As the average population density in the whole culture medium increases the density of Collembola in each aggregation is increased. However, the increase is not proportional, and some of the insects are forced to find or found new aggregations, probably in sub-optimal areas. This would have the effect of conserving the resources in the particularly favourable sites and of controlling the population since some Collembola are forced to live in sub-optimal situations.

5. Summary . Zusammenfassung

Experiments were designed to investigate the pattern of aggregation of *Folsomia candida* (Willem) in laboratory cultures. It is shown that aggregation occurs when there are sufficient individuals and when there has been sufficient time for individuals to find suitable locations. The location of food and water are demonstrated to result in large aggregations, whilst the presence of barriers is a contributory factor. Since both the number of aggregations per unit area and the number of insects in an aggregation are positively correlated with the population density, a type III aggregation pattern for this species is suggested.

[Untersuchungen an Populationen von *Folsomia candida* (Insecta, Collembola): Ursachen von Aggregationen]

Experimente wurden konzipiert, um die Art der Aggregation von *Folsomia candida* (Willem) in Laboratoriums-Kulturen untersuchen zu können. Es wurde gezeigt, daß Aggregationen entstehen, wenn genügend Individuen vorhanden sind und wenn diese genügend Zeit hatten, um zusagende Lokalitäten zu finden. Es wurde auch gezeigt, daß das Vorkommen von Nahrung und Wasser große Aggregationen verursacht, während das Vorhandensein von (Struktur-)Barrieren ein zusätzlicher Faktor ist. Wenn beides, die Zahl der Aggregationen per Raumeinheit und die Zahl der Insekten in einer Aggregation, mit der Besatzdichte positiv korreliert ist, wird angenommen, daß es sich um den Typ III des Aggregationsmodus dieser Art handelt.

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